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The occurrence of callose during the process of local lesion formation

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Abstract

Callose deposition was studied in relation to the development of local lesions using various virus-host combinations.

Fluorescent spots due to callose formation at the initial sites of infection could be detected several hours before local lesions appeared in the combinations *Nicotiana glutinosa* – tobacco mosaic virus; 'Samsun NN' tobacco and 'Pinto' bean – tobacco mosaic virus and tobacco necrosis virus; 'Pinto' bean – cowpea mosaic virus. The fluorescent spots enlarged with time, and covered the lesion areas. Following the development of lesions the fluorescence decreased and was then to be seen only around the lesions. It eventually disappeared when the necrosis of cells in the lesions was complete. Strong callose fluorescence was also seen in veins near the lesions. In some cases fluorescence remained after local lesions had turned completely brown.

When 'Samsun NN' tobacco was studied with tomato spotted wilt, tobacco rattle and tomato black ring viruses, which produce systemic necrosis as well as local lesions on the inoculated plants, similar callose fluorescence could be detected prior to local lesion formation. Here also, the fluorescence was clear during the early stages of lesion formation and grew weaker as lesion formation progressed, though in these cases no fluorescence was seen in veins near the lesions.

The relation between callose deposition in and outside the local lesions and the restriction of virus movement from these tissues is discussed.

Introduction

How a virus is localized in a hypersensitive host is not clear. Since Esau (1967) suggested that plasmodesmata are pathways for intercellular movement of virus particles and spread of virus may be prevented by callose deposition in plasmodesmata, several studies showing a positive correlation between callose deposition and the prevention of viral movement have been reported (Wu et al., 1969; Wu and Dimitman, 1970; Hiruki and Tu, 1972; Shimomura, 1972).

We have now studied this further in a number of virus – host combinations.

Materials and methods

Plants and viruses. Nicotiana tabacum 'Samsun NN', *N. glutinosa* and *Phaseolus vulgaris* 'Pinto' were used as the local lesion hosts, and *N. tabacum* 'Samsun' as the systemic host of tobacco mosaic virus (TMV). 'Samsun NN' was also used as the local lesion host of tobacco necrosis virus (TNV), tomato spotted wilt virus (TSWV), tobacco rattle virus (TRV), and tomato black ring virus (TBRV). 'Pinto' bean was also used as the local lesion host of TNV and cowpea mosaic virus (CpMV). *Petunia hybrida* 'Vilmorin' was the local lesion host of TSWV. Tobacco, *N. glutinosa*, and *Petunia* plants

were grown under ordinary greenhouse conditions for 2 months after sowing, and 'Pinto' bean plants were grown for 10–14 days after sowing. Detached leaves inoculated with virus were placed on wet filter paper in petri dishes and incubated at 19 °C, excepting 'Samsun NN' tobacco or *N. glutinosa* leaves inoculated with TMV and incubated at 23 °C, under continuous illumination from fluorescent lamps. Leaf samples were taken at different times after inoculation and examined for callose deposition.

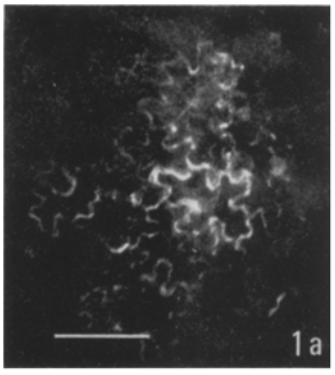
A purified preparation (1 μg/ml) of the common strain of TMV was used. Other virus inocula were prepared by grinding leaves of infected 'Samsun NN' tobacco plants (except in the case of CpMV which was propagated in cowpea plants) with a pestle in a mortar with 0.1 M phosphate buffer, pH 7.0. Inoculations were made by rubbing with the forefinger on the upper surface of leaves previously dusted with carborundum.

Detection of callose. Leaf tissues, about 2 × 3 cm, cut from the leaf samples taken at different times after inoculation, were decolorized with 95% alcohol after 3–4 min immersion in boiling water (Wu et al., 1969). They were then stained with 0.1% aniline blue in 1/15 M K_3PO_4 (pH 12.0) for 1–2 h at room temperature. Observations were made with a Wild microscope with a high pressure mercury vapour lamp (HBO 200W). Ultraviolet transmission filters UG 1 and BG 38, with maximum transmission at 366 nm, together served to absorb the visible spectrum.

Enzymatic digestion of callose by β-1,3-glucan hydrolase. Enzymatic digestion tests using β-1,3-glucan hydrolase were conducted on free-hand sections of healthy *N. glutinosa* petioles and 'Samsun NN' leaf samples with local lesions 40 h after TMV inoculation. Free-hand sections of *N. glutinosa* petioles were treated directly with enzyme solution and 'Samsun NN' leaves were treated with enzyme after boiling in water and decolorization in alcohol. After treatment with enzyme solution, the samples were stained with aniline blue solution and observed under a microscope. One part of the sections was not treated with enzyme solution, but only stained with aniline blue solution and served as control. β-1,3-Glucan hydrolase from Kirin Brewery Co., Ltd., Japan (named zymolyase by Kitamura and Yamamoto, 1972) was used in these experiments. The reactions were performed by adding 5 ml of enzyme solution (0.2% solution in 0.05% phosphate buffer, pH 6.5) to a test tube containing test samples and the test tube was incubated for 30 min in a water bath at 45 °C.

Fig. 1–6. Fluorescence at the site of infection in a *Nicotiana glutinosa* leaf inoculated with tobacco mosaic virus (TMV). (1a–c) 20 h after inoculation; walls of epidermis fluorescent. Bar represents 100 μm, also in the following figures. (2) 24 h after inoculation; fluorescence extended into mesophyll. (3) 30 h after inoculation. (4) 2 days after inoculation; fluorescence more or less disappeared from local lesions but showing up in peripheral zone of the lesions. (5) 3 days after inoculation; no fluorescence in epidermis or mesophyll in and outside the lesions. (6) 6 days after inoculation; again fluorescence in peripheral zone. n = necrotic cells.

Fig. 1–6. Fluorescentie op de plaats van infectie in een blad van Nicotiana glutinosa, geïnoculeerd met tabaksmozaïekvirus (TMV). (1a–c) 20 uur na inoculatie; wanden van epidermis fluorescerend. Het vergrotingsstreepje geeft in alle figuren 100 μm weer. (2) 24 uur na inoculatie; fluorescentie uitgebreid over mesofyl. (3) 30 uur na inoculatie, (4) 2 dagen na inoculatie; fluorescentie min of meer verdwenen uit de lokale lesies maar verschenen in de periferie van de lesies. (5) 3 dagen na inoculatie; geen fluorescentie in epidermis of mesofyl binnenin of aan de buitenkant van de lesies. (6) 6 dagen na inoculatie; weer fluorescentie in de periferie. n = necrotische cellen.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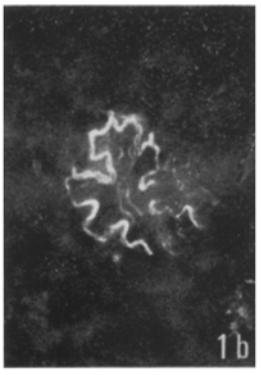
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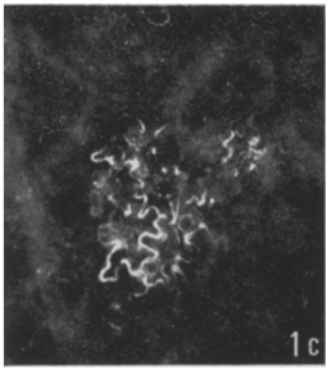
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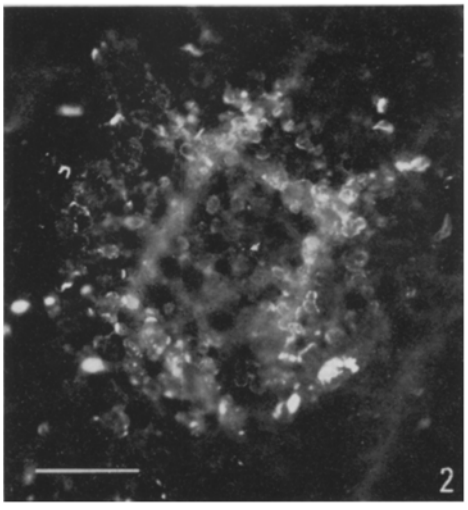
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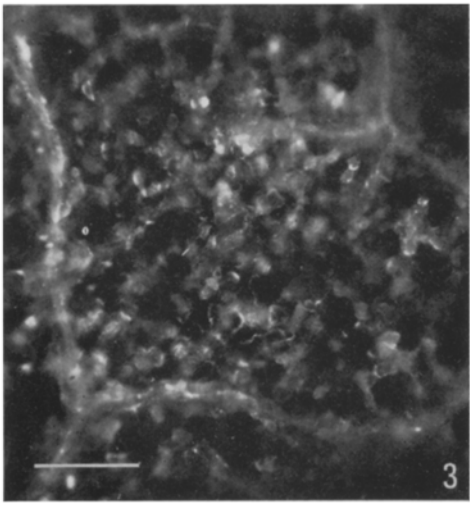
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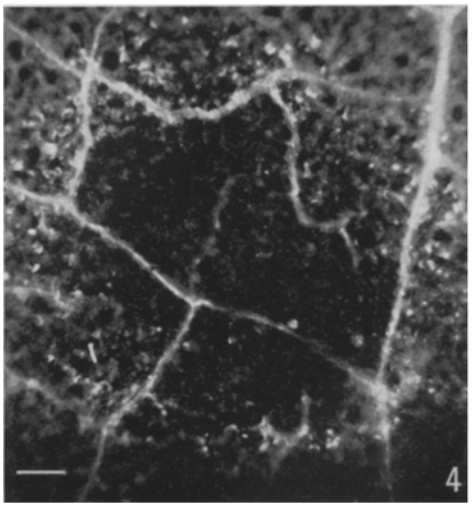
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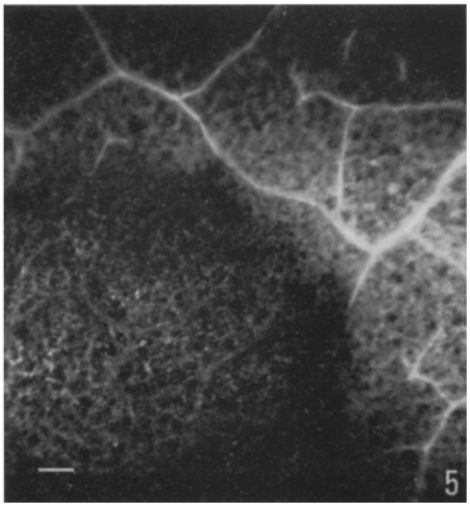
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Results

N. glutinosa leaf with TMV. Twenty hours after inoculation, a few small fluorescent spots could be detected when inoculated leaves were incubated at 23 °C. This callose fluorescence was seen only in peripheral zone of epidermal cells (Fig. 1a–c). Twenty-two hours after inoculation, most of the fluorescent spots at the initial sites of infection were easily detected. At this time, local lesions were not yet detected on the inoculated leaves. Twenty-four hours after inoculation, a few local lesions could be detected as tiny sunken colourless spots, visible only on the lower side of the inoculated leaves. At this stage, fluorescence extended into the mesophyll tissues, enlarged, and covered the lesion areas (Fig. 2, 3). Two days after inoculation, most of the lesions were visible as dark-brown spots on the inoculated leaves. At this time, small lesions still were fluorescent whereas in large lesions fluorescence weakened and was visible mainly at the peripheral zone of the lesions (Fig. 4). Three days after inoculation, the fluorescence was no longer detected in or outside the lesions (Fig. 5). When inoculated leaves were incubated at 19 °C, callose fluorescent spots could be detected 28 h after inoculation and local lesions began to appear 35 h after inoculation.

Dijkstra (1962) reported that local lesions were formed on the stripped areas of *N. glutinosa* leaves from which the TMV-inoculated lower epidermis had been removed 8 h later. We have conducted experiments to determine whether callose can be detected in epidermis-stripped areas of *N. glutinosa* leaves. Lower epidermis was partly removed 16 or 19 h after inoculation with TMV and stripped leaf was incubated at 23 °C. Twenty-three to 26 h after inoculation, clear fluorescent spots, characteristic of TMV lesions, could be detected on stripped areas, though they were smaller than those of epidermis-attached control areas (Fig. 7, 8). Three days after inoculation, this fluorescence was no longer detected.

'Samsun NN' tobacco leaf with TMV. A few small fluorescent spots could be detected 22 h after inoculation, when inoculated leaves were incubated at 23 °C (Fig. 9). Twenty-six hours after inoculation, most fluorescent spots had appeared. The fluorescence was more brilliant than that of *N. glutinosa* leaves (Fig. 10, 11). Twenty-seven hours after inoculation, a few local lesions could be detected. Two days after inoculation, the fluorescence was detectable only in the peripheral zone of the lesions (Fig. 36) and 3 days after inoculation it was no longer detectable, just as in the case of *N. glutinosa* leaf infected with TMV.

In both *N. glutinosa* and 'Samsun NN' tobacco leaves infected with TMV, callose fluorescence around the lesions disappeared 2–3 days after inoculation, as has been described. However, the fluorescence was often observed again in the non-necrotic

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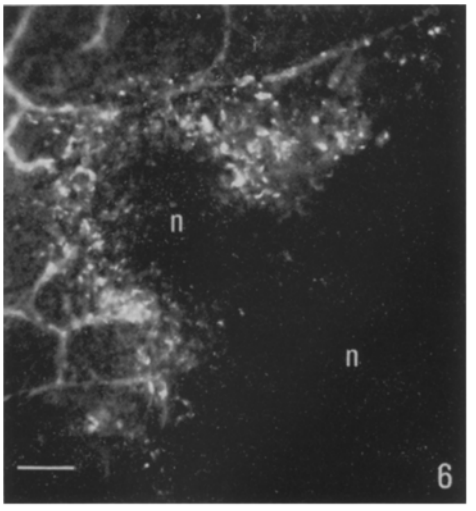
Fig. 7–8. *N. glutinosa* leaf from which the lower epidermis was removed 19 and 16 h, respectively, after inoculation with TMV. Subsequently, the leaf was incubated at 23 °C for 7 h.
Fig. 9–11. Fluorescence in a 'Samsun NN' tobacco leaf inoculated with TMV. (9) 22 h after inoculation. (10–11) 26 h after inoculation; veins show strong fluorescence.

Fig. 7–8. Een N. glutinosa-blad waarvan de onderepidermis resp. 19 en 16 uur na inoculatie met TMV was verwijderd. Het blad was vervolgens gedurende 7 uur geïncubeerd bij 23 °C.
Fig. 9–11. Fluorescentie in een blad van 'Samsun NN'-tabak, geïnoculeerd met TMV. (9) 22 uur na inoculatie. (10–11) 26 uur na inoculatie; sterke fluorescentie van de nerven.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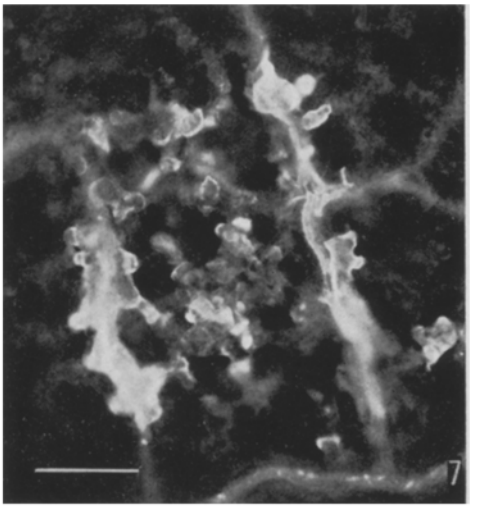
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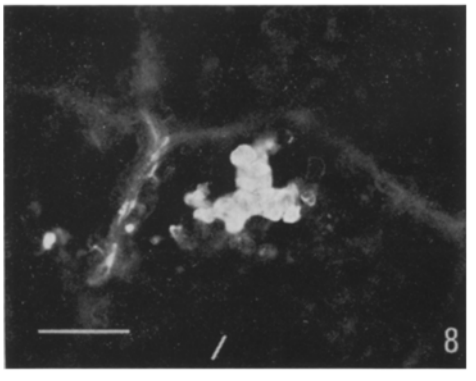
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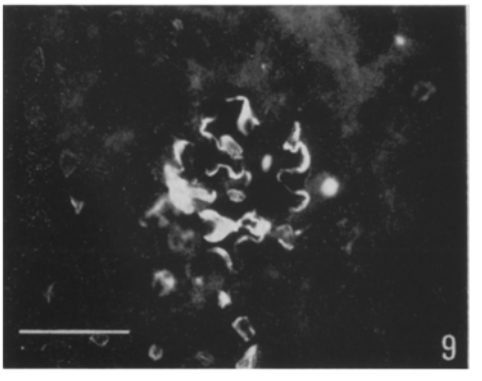
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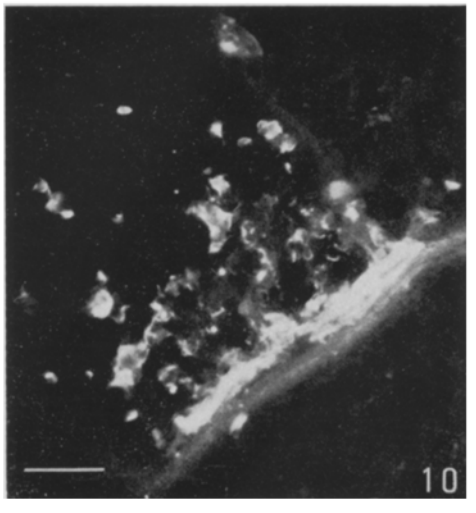
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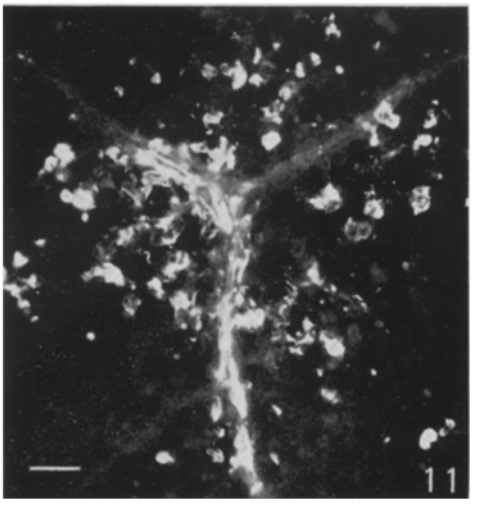
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areas around the necrotic lesions which ceased to enlarge 5–6 days after inoculation (Fig. 6). In such cases, yellowing of the tissue around the lesions was generally observed.

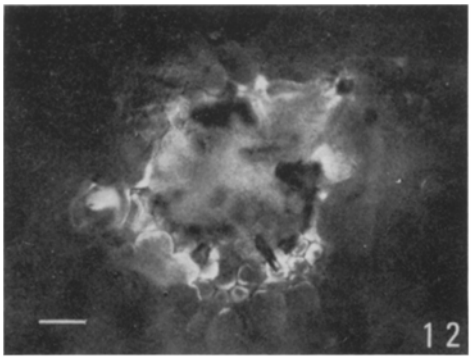
When the experiments were conducted with leaves of intact plants of 'Samsun NN' tobacco or *N. glutinosa*, the time of appearance of local lesions was delayed and the rate of enlargement decreased compared with local lesion development on detached leaves. In TMV-inoculated leaves of intact plants of 'Samsun NN', intensive callose fluorescence in and around the local lesions tended not to disappear even after infected leaves began yellowing (Fig. 12). However, in the case of *N. glutinosa* leaves similarly inoculated with TMV, callose fluorescence always disappeared 2–3 days after inoculation in the same way as with detached leaves.

'Samsun' tobacco leaf with TMV. To determine whether callose deposition in and outside the local lesions on the leaves of a local lesion host is due to the virus infection or to some specific local lesion reaction, experiments were conducted using TMV and its systemic host 'Samsun' tobacco. No callose deposition could be detected in the leaves of infected 'Samsun' at any time after inoculation.

'Pinto' bean leaf with TMV. At 35 h after inoculation, there was a wide variation of the stage of local lesion formation. Brilliant fluorescent spots prior to local lesion formation (Fig. 13), strong fluorescence around the lesions just formed (Fig. 14), and fluorescence around the necrotic lesions (one lesion contained 5–10 necrotic epidermal cells, Fig. 15) were seen. Two days after inoculation, the fluorescence was weaker than 35 h after. Four days after inoculation, faint callose fluorescence was seen on the cell wall at the edge of the seminecrotic zone of the lesion (Fig. 16).

Fig. 12. Local lesion formed on an intact leaf of a 'Samsun NN' tobacco plant, 5 days after inoculation with TMV; strong fluorescence all over the lesion still present.
Fig. 13–16. 'Pinto' bean leaf, inoculated with TMV. (13–15) 35 h after inoculation; wide variation of the stage of local lesion formation. (13) Fluorescent spot appeared before local lesion formation. (14) Fluorescence surrounding the lesion which has just appeared. (15) Fluorescence around the necrotic local lesion. (16) 4 days after inoculation; faint fluorescence on the cell walls at the edge of the seminecrotic zone of the lesion.
Fig. 17–18. 'Samsun NN' tobacco leaf, inoculated with tobacco necrosis virus (TNV). (17) 26 h after inoculation. (18) 3 days after inoculation; fluorescence of the veins adjacent to the lesion (n).
Fig. 19. 'Pinto' bean leaf, 42 h after inoculation with TNV; fluorescence of the veins near the lesions (n).

Fig. 12. Lokale lesie op een intact blad van 'Samsun NN'-tabak, 5 dagen na inoculatie met TMV; nog sterke fluorescentie over de gehele lesie.
Fig. 13–16. Blad van 'Pinto'-boon, geïnoculeerd met TMV. (13–15) 35 uur na inoculatie; grote variatie in de ontwikkelingsstadia van de lokale lesies. (13) Fluorescerend plekje verscheen vóór de vorming van lokale lesies. (14) Fluorescentie rondom de lesie, die zich juist heeft ontwikkeld. (15) Fluorescentie rondom de necrotische lokale lesie. (16) 4 dagen na inoculatie; zwakke fluorescentie op de celwanden op de rand van de half-necrotische zone van de lesie.
Fig. 17–18. Blad van 'Samsun NN'-tabak, geïnoculeerd met tabaksnecrosevirus (TNV). (17) 26 uur na inoculatie. (18) 3 dagen na inoculatie; fluorescentie van de nerven, grenzend aan de lesie (n).
Fig. 19. Blad van 'Pinto'-boon, 42 uur na inoculatie met TNV; fluorescentie van de nerven in de nabijheid van de lesies (n).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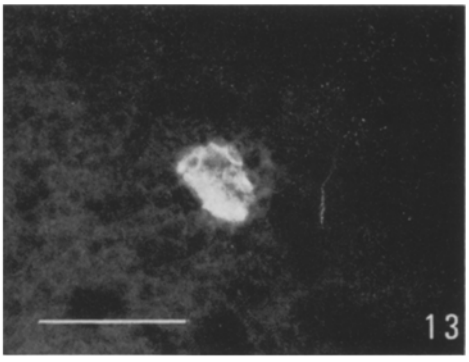
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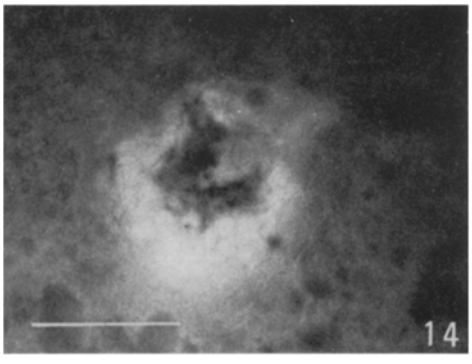
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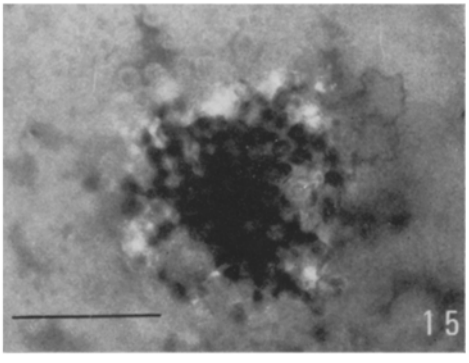
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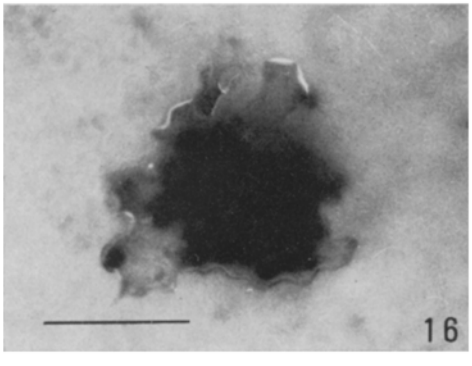
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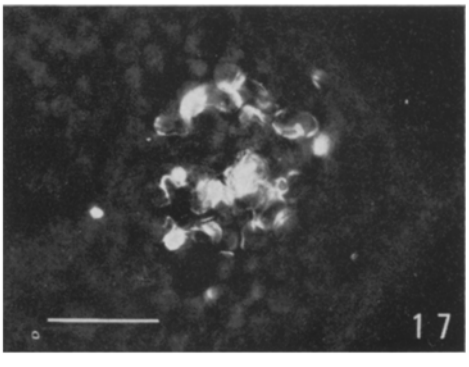
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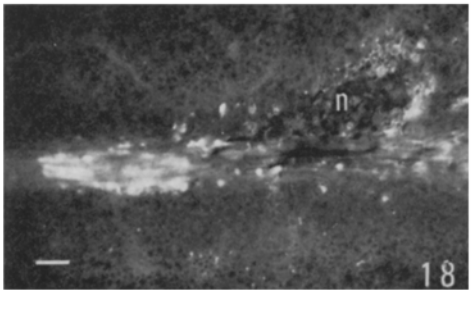
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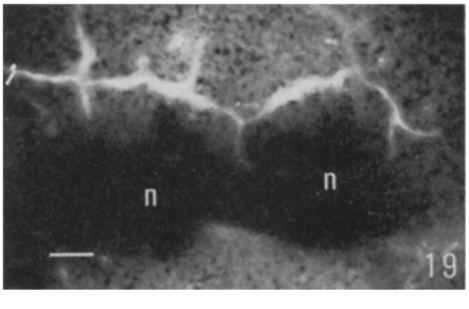
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'Samsun NN' tobacco or 'Pinto' bean leaf with TNV. Local lesions appeared on the leaves of 'Samsun NN' and 'Pinto' bean about 1 and 2 days, respectively, after inoculation. In both plants, fluorescent spots could be detected several hours before local lesion formation (Fig. 17). The fluorescence is most intense during the early stages of lesion formation and disappeared 3 days after inoculation.

'Pinto' bean leaf with CpMV. Strong fluorescence appeared only in the veins connected with the initial sites of infection 40 h after inoculation (Fig. 20). These veins appeared somewhat brown when the stained tissues were observed under the ordinary light microscope. Three days after inoculation, local lesions became visible (veins remarkably browned and fluorescence only in these areas), but fluorescence was no longer detected a week after inoculation.

Callose deposition in the veins near the lesions. In all the above virus-host combinations, intensive callose fluorescence was seen in the veins near the lesions formed on the inoculated leaves (Fig. 2, 3, 4, 8, 10, 11, 20). In the case of 'Samsun NN' tobacco or 'Pinto' bean with TMV (Fig. 21, 22, 23, 36) or TNV (Fig. 18, 19), strong fluorescence of the veins persisted after local lesions had become completely brown.

'Samsun NN' tobacco leaf with TSWV, TRV or TBRV. Tomato spotted wilt virus, TRV and TBRV produce systemic necrosis in the leaves of 'Samsun NN'. Lesions caused by TSWV appeared after 28 h, TRV lesions after 35 h, and TBRV lesions on the third day. All these viruses produced fluorescent spots on the inoculated leaves several hours before local lesions were formed (Fig. 24, 28, 31). The fluorescence was clear during lesion formation and gradually disappeared as with other virus-host combinations. In the leaves infected with TSWV or TBRV, however, seminecrotic tissues were formed around the necrotic areas and they fluoresced intensively (Fig. 26, 27, 29, 30). As already described, such fluorescence was also seen in the veins near the lesions on the leaves inoculated with viruses which produce only local lesions. With TSWV,

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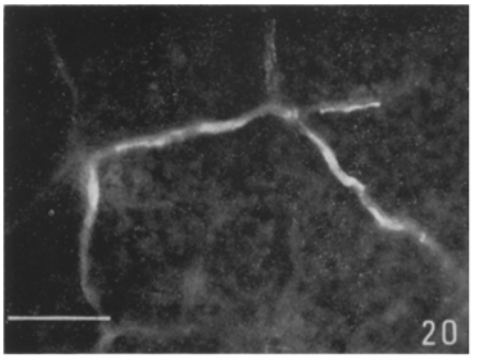
Fig. 20. Lower side of a 'Pinto' bean leaf, 40 h after inoculation with cowpea mosaic virus; fluorescence of the veins.
Fig. 21–22. 'Samsun NN' tobacco leaves, 2 days after inoculation with TMV; fluorescence of the veins in and outside the lesions (n).
Fig. 23. Lower side of a 'Pinto' bean leaf, 42 h after inoculation with TMV; fluorescence of the veins near the lesions.
Fig. 24–27. Fluorescence in a 'Samsun NN' tobacco leaf, inoculated with tomato spotted wilt virus. (24) 24 h after inoculation. (25) 2 days after inoculation. (26) 3 days after inoculation; fluorescence in the seminecrotic tissues around the lesion. (27) 4 days after inoculation. n = necrotic cells.

Fig. 20. Onderkant van een blad van 'Pinto'-boon, 40 uur na inoculatie met 'cowpea'-mozaïekvirus; fluorescentie van de nerven.
Fig. 21–22. Bladeren van 'Samsun NN'-tabak, 2 dagen na inoculatie met TMV; fluorescentie van de nerven binnenin en aan de buitenkant van de lesies (n).
Fig. 23. Onderkant van een blad van 'Pinto'-boon, 42 uur na inoculatie met TMV; fluorescentie van de nerven in de nabijheid van de lesies.
Fig. 24–27. Fluorescentie in een blad van 'Samsun NN'-tabak, geïnoculeerd met tomatebronsvlekkenvirus. (24) 24 uur na inoculatie. (25) 2 dagen na inoculatie. (26) 3 dagen na inoculatie; fluorescentie in de half-necrotische weefsels rondom de lesie. (27) 4 dagen na inoculatie. n = necrotische cellen.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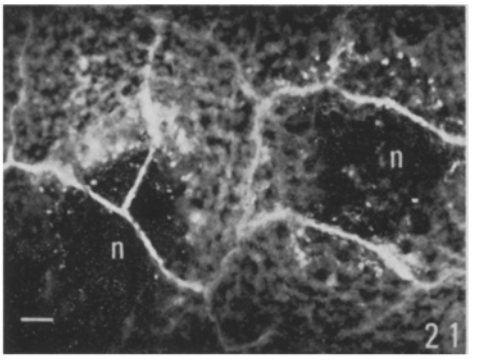
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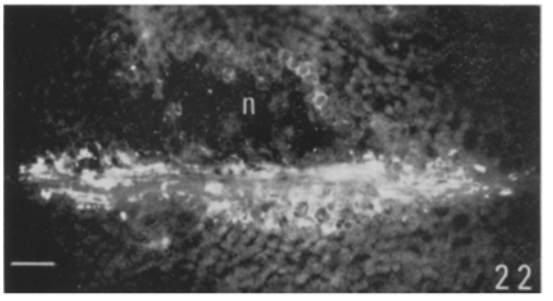
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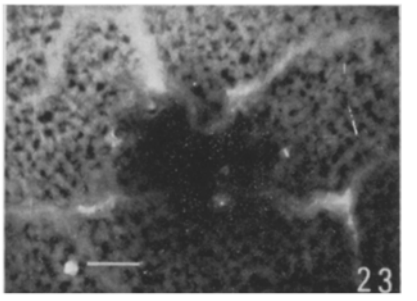
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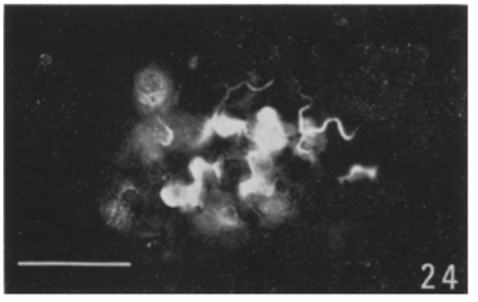
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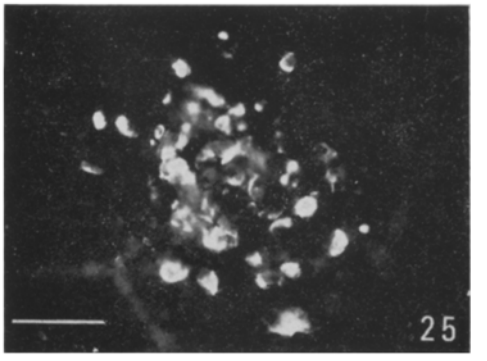
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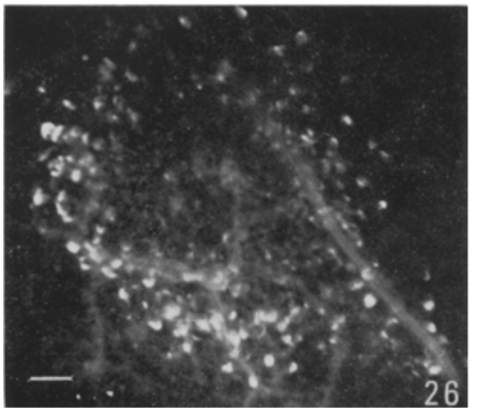
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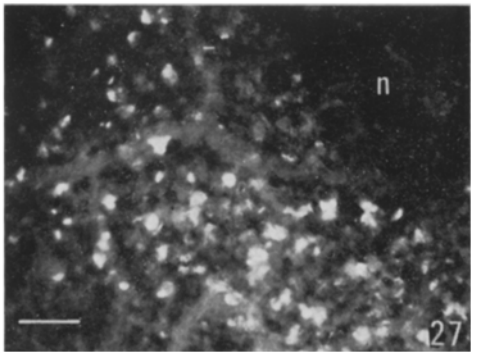
n
27

Fig. 28–30. Fluorescence in a 'Samsun NN' tobacco leaf, inoculated with tomato black ring virus. (28) 60 h after inoculation. (29) 3 days after inoculation; fluorescence in the seminecrotic tissues around the lesion. (30) 4 days after inoculation. n = necrotic cells.
Fig. 31–32. Fluorescence in a 'Samsun NN' tobacco leaf inoculated with tobacco rattle virus. (31) 30 h after inoculation. (32) 40 h after inoculation.

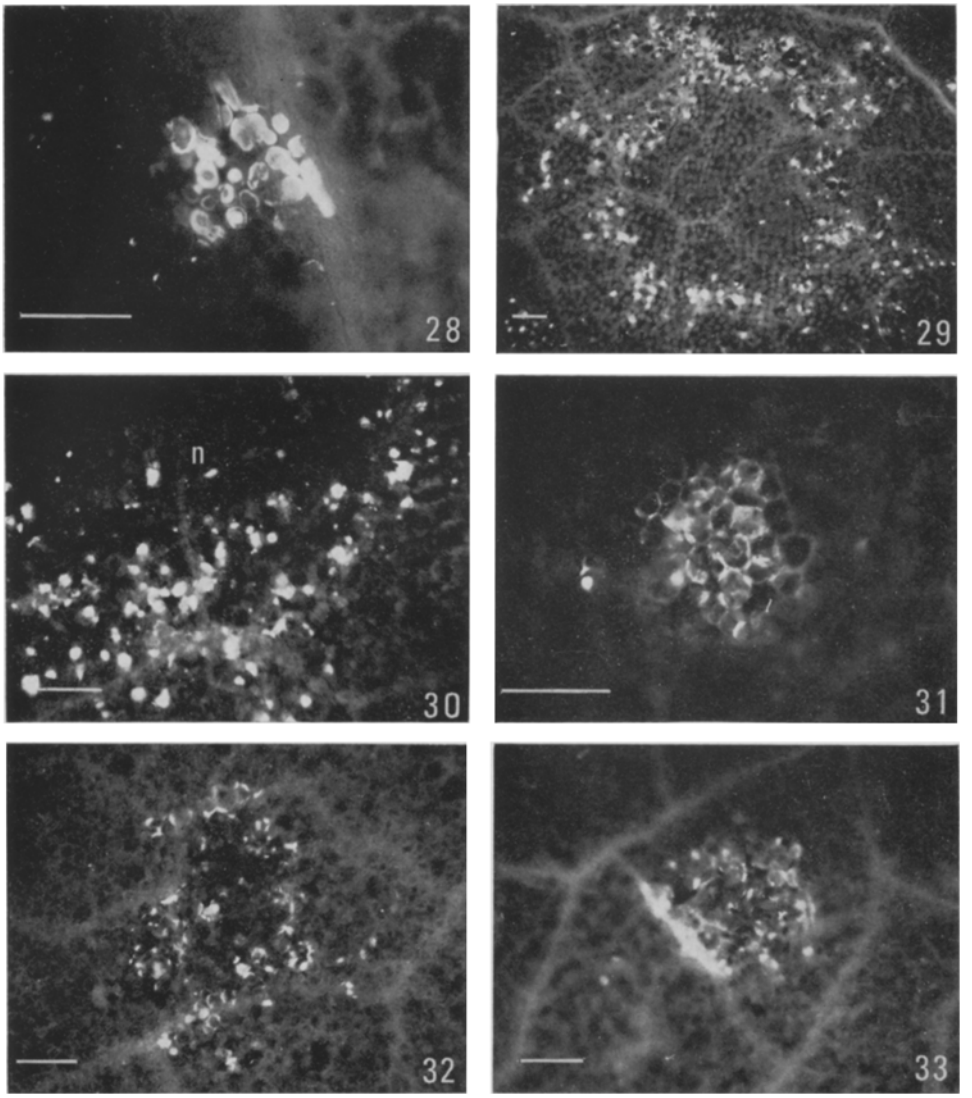

Fig. 33. *Petunia* leaf, 4 days after inoculation with tomato spotted wilt virus.
Fig. 28–30. Fluorescentie in een blad van 'Samsun NN'-tabak, geïnoculeerd met zwarte-kringenvirus van tomaat. (28) 60 uur na inoculatie. (29) 3 dagen na inoculatie; fluorescentie in de half-necrotische weefsels rondom de lesie. (30) 4 dagen na inoculatie. n = necrotische cellen.
Fig. 31–32. Fluorescentie in een blad van 'Samsun NN'-tabak, geïnoculeerd met tabaksratelvirus. (31) 30 uur na inoculatie. (32) 40 uur na inoculatie.
Fig. 33. Blad van Petunia, 4 dagen na inoculatie met tomatebronsvlekkenvirus.

TRV and TBRV, however, no callose fluorescence was produced in the veins near the lesions of 'Samsun NN' (Fig. 25, 26, 27, 29, 30, 32).

When *Petunia* leaves were inoculated with TSWV, only local lesions appeared 40 h after inoculation. The lesions turned brown when they were still small (< 0.2 mm), so it was rather difficult to detect the callose. In some cases, however, the lesions were still fluorescent 40 h after inoculation, and intensive fluorescence was seen in the veins near the lesions even 4 days after inoculation (Fig. 33).

Disappearance of fluorescence by β-1,3-glucan hydrolase. To test whether the fluorescence around the lesions is due to callose, zymolyase was used for enzymatic digestion. Fluorescence in cross sections of the phloem of healthy *N. glutinosa* petioles (Fig. 34) disappeared after enzyme treatment (Fig. 35). The fluorescence around the lesions on

Fig. 34–35. Cross section of a petiole of a healthy *N. glutinosa* plant. (34) Fluorescence, caused by callose, in the phloem. (35) After treatment with β-1,3-glucan hydrolase; most of the fluorescence disappeared.
Fig. 36–37. 'Samsun NN' tobacco leaf, 40 h after inoculation with TMV. (36) Strong fluorescence around the lesion. (37) After treatment with β-1,3-glucan hydrolase; all the fluorescence disappeared.

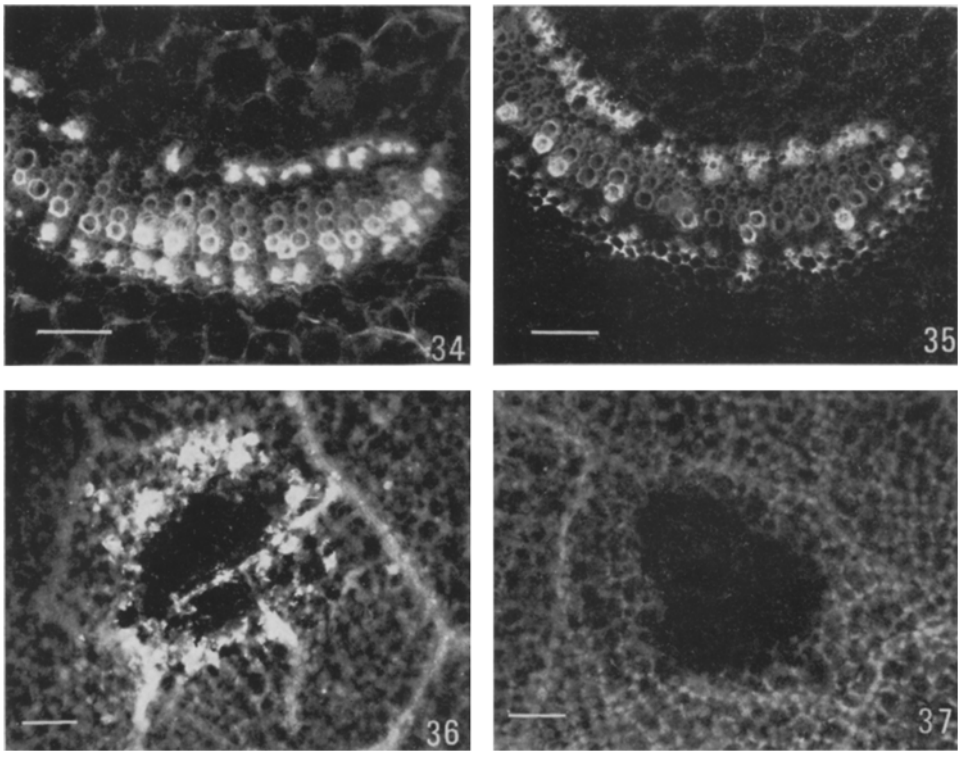

Fig. 34–35. Dwarsdoorsnede door een bladsteel van een gezonde N. glutinosa-plant. (34) Fluorescentie in het floëem als gevolg van de aanwezigheid van callose. (35) Na behandeling met β-1,3-glucan hydrolase; fluorescentie grotendeels verdwenen.
Fig. 36–37. Blad van 'Samsun NN'-tabak, 40 uur na inoculatie met TMV. (36) Sterke fluorescentie rondom de lesie. (37) Na behandeling met β-1,3-glucan hydrolase; alle fluorescentie is verdwenen.

the 'Samsun NN' leaves 40 h after inoculation with TMV (Fig. 36) also disappeared after treatment with enzyme (Fig. 37).

Discussion

Wu et al. (1969) reported that callose was deposited on the walls adjacent to a necrotic lesion produced by TMV-U_1 on a 'Pinto' bean leaf 4 days after inoculation. Their data indicated that the deposition in epidermal cells 2–3 days after inoculation seemed weaker than after 4 days and no fluorescence could be detected before necrosis occurred. Hiruki and Tu (1972) also reported that callose was detectable in a zone of non-necrotic cells at the margin of lesions produced by potato virus M on the leaf of *Phaseolus vulgaris* 'Red Kidney' 3 to 4 days after inoculation. They also could not detect fluorescence in inoculated leaves before the lesions developed. Spencer and Kimmins (1971), in electron microscopic studies, were unable to detect the presence of callose in 'Pinto' bean leaf 3 days after inoculation with TMV-U_1. Simon et al. (1972) in ultrastructural studies were also unable to detect callose deposits anywhere in 'Samsun NN' tobacco 7 days after inoculation with TMV.

In contrast to these investigations, the present work has shown that prominent callose deposits can be detected before local lesion formation in various virus and host combinations. The characteristic fluorescence weakened with progressive browning of the tissue, and disappeared when the tissue became completely necrotic (3–4 days after inoculation). Absence of the fluorescence in necrotic tissues may be due to decomposition of the callose rather than to a difficult staining with aniline blue; Moore and Stone (1972) reported that localized infection with TMV or TSWV led to an increase in β-1,3-glucan hydrolase both in and around the lesions produced on the *N. glutinosa* leaf. Faulkner et al. (1973) stated that aniline blue may not be specific as a fluorochrome for β-1,3-glucans. In this investigation, a bright yellow fluorescence in and around the local lesion was considered to indicate the presence of callose because this fluorescence could not be detected in the preparation which had been treated with β-1,3-glucan hydrolase.

In 'Samsun' tobacco systemically infected with TMV, the fluorescence characteristic of TMV infection on 'local lesion type' plants was not detected in inoculated leaves at any time after inoculation. Thus, the callose deposition in the leaf with local symptoms may not be due to virus infection, but to local lesion formation. Callose was detected early during local lesion formation and its characteristic fluorescence grew weaker as tissue browning progressed, usually disappearing entirely when the necrosis was complete. If callose plays a rôle in restricting virus movement it must happen at an early stage of the infection, since once the virus invades veins of inoculated leaves, it rapidly spreads through the entire vascular system. After necrosis of local lesions is complete, acquired resistance (Ross, 1961a, b), secondary cell wall thickening (Tu and Hiruki, 1971; Hiruki and Tu, 1972), or inhibitory substances (Loebenstein and Ross, 1963; Sela et al., 1966; Kimmins, 1969) may further restrict the movement of virus. Callose, normally present in healthy tissue, seems to be formed during the initial stages of infection as a general host reaction to cell injury, and may possibly more rapidly inhibit virus movement than other inhibitory factors. Very often, 5–6 days after inoculation, fluorescence was redetected in the non-necrotic cells surrounding necrotic lesions that had ceased to enlarge. Although callose was still being formed around such lesions,

even after they had become completely necrotic, it was apparently being decomposed very quickly after it was formed. When local lesions did not further enlarge 5–6 days after inoculation and more callose was being formed than decomposed, the callose accumulated again around the lesions.

With virus and host combinations producing *systemic* necrosis in the inoculated leaves, intensive fluorescence was seen during local lesion formation. Especially with TSWV or TBRV in 'Samsun NN' tobacco, strong fluorescence was always detected in the semi-necrotic zone around the necrotic tissues. It is not clear why the virus becomes systemic in such cases, regardless of prominent callose accumulation in or around the local lesions formed. In our experiments, no fluorescence of the veins near the lesions was detected in inoculated leaves showing systemic necrosis, although strong fluorescence was noted with virus-host combinations producing non-systemic necrosis. These results suggest that prominent callose deposition in the veins or outside lesions may lead to a sealing off of the veins and to early death of the clogged part of the veins, thus preventing the systemic spread of virus. However, it cannot be excluded that factors other than callose deposition are involved in the restriction of virus movement.

Samenvatting

Het optreden van callose gedurende het proces van lokale-lesievorming

Met behulp van fluorescentiemicroscopie werd de relatie tussen calloseafzetting en lokale-lesievorming onderzocht bij verschillende virus-waardplantcombinaties.

De combinaties tabaksmozaïekvirus (TMV) – *Nicotiana glutinosa*, TMV en tabaksnecrosevirus (TNV) – *Nicotiana tabacum* 'Samsun NN' en *Phaseolus vulgaris* 'Pinto', alsmede 'cowpea'-mozaïekvirus – *P. vulgaris* 'Pinto' gaven na inoculatie van de bladeren hierop fluorescerende plekjes te zien als gevolg van callosevorming gedurende de eerste stadia van infectie. Deze plekjes konden reeds enige uren voordat lokale lesies verschenen waargenomen worden (Fig. 1, 2, 3, 9, 10, 11, 13, 17). Proeven, waarbij een deel van de epidermis van *N. glutinosa*-bladeren 16–19 uur na inoculatie met TMV was verwijderd, wezen uit dat ook in dit geval fluorescerende plekjes optraden, die echter kleiner waren dan die, op de bladgedeelten die nog in het bezit waren van de epidermis (Fig. 7, 8). Bij 'Pinto'-boon met 'cowpea'-mozaïekvirus was alleen sterke fluorescentie te zien in de nerven op de oorspronkelijke plaats van infectie, vóór het verschijnen van de lokale lesies (Fig. 20).

Na aanvankelijke uitbreiding (Fig. 14, 15) nam het gebied van fluorescentie gedurende de verdere ontwikkeling van de lokale lesies af, was slechts zichtbaar rondom de lesies (Fig. 4, 16, 36) en verdween geheel als de necrose van de cellen in de lesies was voltooid (Fig. 5). Een uitzondering vormden 'Samsun-NN'-tabak en *N. glutinosa* met TMV als met intacte bladeren werd gewerkt; in dat geval verdween de fluorescentie zelfs niet bij ouder worden van de lesies (Fig. 12). Bij laatstgenoemde combinaties werd in geval van afgeknipte bladeren bij verdere veroudering van de lesies soms opnieuw fluorescentie in het niet-necrotische, vaak vergeelde weefsel rondom de necrotische lesies waargenomen (Fig. 6).

Naast fluorescentie op de plaats van infectie was er ook sterke fluorescentie zichtbaar in de nerven in de nabijheid van de lesies (Fig. 2, 3, 4, 8, 10, 11, 20). Bij 'Samsun NN'-tabak en 'Pinto'-boon met TNV en TMV handhaafde de fluorescentie in de ner-

ven zich zelfs nadat de lokale lesies volledig bruin waren geworden (Fig. 18, 19, 21, 22, 23, 36).

In 'Samsun NN'-tabak met tomatebronsvlekkenvirus, tabaksratelvirus en zwarte-kringenvirus van tomaat, waarbij de planten alle tevens met systemische necrose reageren, trad er, evenals bij de eerder genoemde combinaties van slechts lokaal-reagerende planten, fluorescentie op vóór de vorming van lokale lesies (Fig. 24, 28, 31). Ook in deze gevallen nam de fluorescentie af naarmate de lesies zich ontwikkelden (met uitzondering van de combinaties met tomatebronsvlekkenvirus en zwarte-kringenvirus van tomaat, waarbij sterke fluorescentie werd waargenomen rondom de necrotische weefsels (Fig. 26, 27, 29, 30), maar niet in de nerven (Fig. 25, 26, 27, 29, 30, 32).

Bij *Petunia*, die ongeveer 40 uur na inoculatie met tomatebronsvlekkenvirus lokale lesies geeft, bleef fluorescentie in de lesie bestaan en na 4 dagen was deze zelfs sterker in de nerven in de nabijheid van de lesies (Fig. 33).

Om er zeker van te zijn dat het de callose rondom de lesies is die fluoresceert, werden bladmonsters van *N. glutinosa* met TMV behandeld met zymolyase (β-1,3-glucan hydrolase). De fluorescentie in dwarsdoorsneden van bladstelen van gezonde *N. glutinosa* (Fig. 34) verdween door behandeling met de enzymoplossing (Fig. 35). Fluorescentie rondom de lesies, gevormd op 'Samsun NN'-tabak, 40 uur na inoculatie met TMV (Fig. 36) verdween eveneens na behandeling met het enzym (Fig. 37).

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Book review

K. Heinze: Leitfäden der Schädlingsbekämpfung, Band I. Schädlinge und Krankheiten im Gemüsebau. Wissensch. Verlagsgesellschaft m.b.H., Stuttgart, 4th edn. 1974. 360 pp, 48 figs, 1629 references, subject index; cloth bound, dust jacket. Price DM 96.

This German book on 'Pests and diseases in vegetable growing' is a completely rewritten text of Volume I of W. H. Frickhinger's 'Guides to pest control', first published some 30 years ago.

It contains concise descriptions of the animal pests, viruses, fungi, and non-parasitic diseases of various crops, with diagnostic data on damage and symtoms caused, and on the pests and disease incitants themselves, their biology and, of course, their control. Weeds are not described, but for each crop specific methods of weed control are listed.

The text has been carefully prepared, is up-to-date and refers to an extensive list of publications. The recording of virus particle sizes in mm instead of in nm (1 mm = 1,000,000 nm) is uncommon and cumbersome.

The book has been written especially for Germany, as shown by the language used and the selection of crops, pests and diseases included. However, it provides much valuable information for surrounding countries in Western and Central Europe. It will be of great help to commercial growers with a scientific background, to advisory officers, teachers, students and all others involved in plant protection.

L. Bos

H. G. Fransz: The functional response to prey density in an acarine system. Pudoc, Wageningen, 1974. 143 pp, 32 figs, 4 plates. Paperback. Price Dfl. 20.80.

The effect of prey density on the number of prey killed, (the functional response curve), was the subject of Fransz' thesis. In a careful study he succeeded in quantifying the many behavioural components involved in the predation process. This enabled him to construct a simulation model with a new method of simulation, the compound simulation. He showed that the shape of the computed functional response curve is mainly determined by the decrease of the success ratio with increasing prey density. The simulation showed that in biological control it can be advantageous to introduce a harmless and rather unattractive alternative prey species together with the predator.

The book is considered as a most useful contribution to the understanding of prey-predator relationships.

G. W. Ankersmit